FORESTRY

FRDA REPORT 175

SIVE, A New Stock Quality Test: The First Approximation

ISSN 0835 0752

JANUARY 1992

CANADA-BRITISH COLUMBIA PARTNERSHIP AGREEMENT ON FOREST RESOURCE DEVELOPMENT: FRDA II

Canada

BC

SIVE, A New Stock Quality Test: The First Approximation

by

C.D.B. Hawkins[1] and D.R. DeYoe[2]

[1] Land Use Planning Advisory Team
MacMillan Bloedel Limited
Woodlands Services Division
65 Front Street
Nanaimo, B.C. V9R 5H9

[2] Ontario Ministry of Natural Resources
258 Queen Street East
P.O. Box 1000
Sault Ste. Marie, Ontario P6A 5N5

January 1992

CANADA-BRITISH COLUMBIA PARTNERSHIP AGREEMENT ON FOREST RESOURCE DEVELOPMENT: FRDA II

Canada

BC

Funding for this publication was provided by the Canada-British Columbia Partnership Agreement on Forest Resource Development: FRDA II – a four year (1991-95) $200 million program cost-shared equally by the federal and provincial governments.

Canadian Cataloguing in Publication Data

Hawkins, Christopher David Borden.
SIVE, a new stock quality test

(FRDA report, ISSN 0835–0752 ; 175)

"Canada-British Columbia Partnership Agreement on Forest Resource Development: FRDA II."
Co-published by B.C. Ministry of Forests.
Includes bibliographical references: p.
ISBN 0–7726–1505–5

1. Spruce – British Columbia – Prince George Region – Frost resistance. 2. Douglas-fir – British Columbia – Prince George Region – Frost resistance. 3. Spruce – British Columbia – Prince George Region – Effect of stress on. 4. Douglas-fir – British Columbia – Prince George Region – Effect of stress on. 5. Spruce – British Columbia – Prince George Region – Seedlings – Quality. 6. Douglas-fir – BritishColumbia – Prince George Region – Seedlings – Quality. I. DeYoe, D. R. (David R.) II. Canada. Forestry Canada. III. Canada-British Columbia Partnership Agreement on Forest Resource Development: FRDA II. IV. British Columbia. Ministry of Forests. V. Title. VI. Series.

SD397.C7.H38 1992 634.9'75256 C92–092089–6

© 1992 Government of Canada,
Province of British Columbia

This is a joint publication of Forestry Canada
and the British Columbia Ministry of Forests.

For additional copies and/or further information about the Canada-British Columbia Partnership Agreement on Forest Resource Development: FRDA II, contact:

Forestry Canada
Pacific Forestry Centre
506 West Burnside Road
Victoria, B.C. V8Z 1M5
(604) 363-0600

or

B.C. Ministry of Forests
Forest Science Research Branch
31 Bastion Square
Victoria, B.C. V8W 3E7
(604) 387-6719

93-00509/

SUMMARY

In this report, the SIVE (stress-induced volatile emissions) technique is compared to other, more conventional, stock quality tests used to assess low temperature damage in stored and planted spruce seedlings. The findings show that SIVE compares favorably to the other methodologies. Its results are conservative, and are available in hours rather than days or weeks. SIVE data at the time of freezing was representative of the end-of-season survival of Sxs spruce and correlated significantly to it.

SIVE was also used to follow seasonal baseline gas production of control and drought-treated Douglas-fir seedlings in the nursery. SIVE, via the gas ethanol, was able to distinguish between the two nursery treatments. It appears that an operational nursery seedling vigor screening assessment can be developed with the use of a single gas: ethanol.

Seasonal assessments of Douglas-fir frost resistance, comparing SIVE to other, more conventional methodologies, were also done. According to SIVE and visual assessments, Douglas-fir frost hardiness induction goes through three phases before reaching the winter hardiness phase. Preliminary critical thresholds of seasonal ethanol production in response to stress were established in this study. Ethanol and acetaldehyde production correlated significantly to foliage browning, as did the electrical conductivity damage ratio. Ethanol production also correlated significantly to the electrical conductivity damage ratio. Again, SIVE data compared favorably to existing, accepted assessment methods.

The next steps in SIVE development are to establish an ethanol-based screening technique that can be used in both the laboratory and field situations; to refine the present analytical system so it backs up the screening technique; and to continue expanding the data base to other species and stress assessments.

ACKNOWLEDGMENTS

We wish to thank the staff of the Land Use Planning Advisory Team, Forest Sciences Laboratory, for their excellent technical assistance, and Glen Dunsworth for having time for discussions and comments on the report. This project was funded under the Forest Resource Development Agreement (FRDA), Direct Delivery Project F-52-41-008. D.R. Drakeford is thanked for input at early stages of this project. Spruce seedlings were supplied by the B.C. Ministry of Forests, Red Rock Research Station, Prince George. This report has been reviewed by Forestry Canada and approved for distribution. This approval does not necessarily signify that the contents reflect the views or policies of Forestry Canada.

TABLE OF CONTENTS

SUMMARY iii

ACKNOWLEDGMENTS iv

1 INTRODUCTION 1

2 MATERIALS AND METHODS 1

2.1 Experiment 1 1
2.1.1 Pre-planting assessments 2
Freezing 2
SIVE sampling 2
Electrical conductivity 2
Visual assessments and survival 3
2.1.2 Post-planting assessments 3
2.1.3 Statistical analysis 4
2.2 Experiment 2 5
2.2.1 Douglas-fir seasonal baseline 5
2.3 Experiment 3 5
2.3.1 Douglas-fir response to low temperature 5

3 RESULTS AND DISCUSSION 5

3.1 Experiment 1: Pre-planting Assessments 5
3.2 Experiment 1: Post-planting Assessments 8
3.3 Experiment 2: Seasonal Baseline in Douglas-fir 10
3.4 Experiment 3: Response to Low Temperature in Douglas-fir 15

4 SUMMARY AND CONCLUSIONS 20

5 LITERATURE CITED 21

APPENDICES

1 Modified GC program for acetaldehyde and ethanol 23

2 Mean variable values for significant ANOVA factors 23

3 Significant first-order interactions 24

TABLES

1 ANOVA model used for assessment of pre-planting experimental variables 4

2 ANOVA model used for assessing post-planting experimental variables 4

3 Significance of treatments on ethanol and acetaldehyde production, standardized electrical conductivity, foliage browning, bud damage, cambium damage, and end-of-season survival in Sxs removed from cold storage and tested 6

4 The effect of temperature on ethanol and acetaldehyde production, standardized electrical conductivity, foliage browning, bud damage, cambium damage, and end-of-season survival in Sxs removed from cold storage and tested 7

5 The effect of photoperiod on standardized electrical conductivity, foliage browning, bud damage, cambium damage, and end-of-season survival in Sxs removed from cold storage and tested 7

6 The effect of photoperiod application duration on standardized electrical conductivity and foliage browning in Sxs 8

7 Significance of treatments on ethanol and acetaldehyde production, standardized electrical conductivity, EC damage ratio, foliage browning, bud damage, cambium damage, and end-of-season survival in Sxs exposed to low temperature stresses at a various number of days after planting 9

8 The effect of temperature on ethanol and acetaldehyde production, standardized electrical conductivity, EC damage ratio, foliage browning, bud damage, cambium damage, and end-of-season survival in Sxs exposed to low temperature stresses at a various number of days after planting 10

9 Regression equation, correlation coefficient, and level of significance for end-of-season survival as a function of ethanol production and foliage browning in Sxs after a post-planting, low temperature stress test 11

10 LT_{25} of foliage, buds, and cambia in nursery control and drought-treated Douglas-fir seedlings from August 1989 through February 1990 18

11 Regression equation, correlation, and level of significance of various parameters during the period of maximum frost hardiness, Phase IV, for control seedlings and nursery drought-treated Douglas-fir seedlings 20

FIGURES

1 End-of-season mean survival versus mean ethanol production for all P×D×T nursery treatments of Sxs spruce exposed to temperature stresses directly on removal from storage 8

2 End-of-season mean survival versus mean foliage browning for all P×D×T nursery treatments of Sxs spruce exposed to temperature stresses directly on removal from cold storage 9

3 Seasonal mean acetaldehyde production of control and drought-treated Douglas-fir grown at the A.P. MacBean Nursery in 1989/1990 11

4 Seasonal mean ethanol production of control and drought-treated Douglas-fir grown in 1989/1990 12

5 Seasonal mean acetaldehyde to ethanol ratio of control and drought-treated Douglas-fir grown in 1989/1990 13

6 Seasonal change of mean standardized electrical conductivity of control and drought-treated Douglas-fir grown in 1989/1990 14

7 Seasonal mean electrical conductivity damage ratio of control and drought-treated Douglas-fir grown at nursery in 1989/1990 14

8 Phase I mean ethanol and acetaldehyde production in Douglas-fir seedlings exposed to the indicated temperatures 15

9 Mean electrical conductivity damage ratio for nursery control and drought-treated Douglas-fir seedlings exposed to the indicated temperatures during Phases I and II 16

10 Phase II mean acetaldehyde production and ethanol in nursery control and drought-treated Douglas-fir seedlings exposed to the indicated temperatures 16

11 Phase III mean acetaldehyde production and ethanol in nursery control and drought-treated Douglas-fir seedlings exposed to the indicated temperatures 17

12 Mean electrical conductivity damage ratio for nursery control and drought-treated Douglas-fir seedlings exposed to the indicated temperatures during Phase III 17

13 Phase IV mean acetaldehyde production and ethanol in nursery control and drought-treated Douglas-fir seedlings exposed to the indicated temperatures 19

14 Mean electrical conductivity damage ratio for nursery control and drought-treated Douglas-fir seedlings exposed to the indicated temperatures during Phase IV 19

1 INTRODUCTION

Stock quality or seedling vigor testing is important at all phases in the forest regeneration process (Sutton 1988; Puttonen 1989; Ritchie 1989). Vigor testing is not new (Sutton 1979). Tests based on seedling physiology are becoming of paramount importance (Duryea 1985) because of their quantitative nature (Hawkins and Binder 1990). For a physiologically based test to be viable, it should meet the seven prerequisites outlined by Zaerr (1985): rapid, simple, accessible, reliable, nondestructive, quantitative and diagnostic. While some conventional tests meet some of these criteria, most fall far short (Hawkins and Binder 1990).

High levels of seedling stress resistance correlate well to seedling survival and growth (Ritchie 1989). Functionally, cold hardiness or frost resistance, a mechanism separate but parallel to dormancy, prepares tissues to withstand winter stresses (Weiser *et al.* 1979; Levitt 1980). This intensifies the seedling's tolerance to a number of interrelated stresses (Levitt 1980; Lavender 1984), but does not preclude it from being stressed.

Conventional frost (stress) resistance assessments of stock rely on visual inspections about 7–10 days after exposure to freezing temperatures (Ritchie 1984). Needles, cambia, buds and roots are often assessed (Glerum 1985). Needles are classed as percentages killed, damaged and unaffected (Colombo and Glerum 1984), while the cambium is scored as to degree and location of injury (Glerum 1985). Buds are usually scored as healthy or damaged. Damage to greater than 50% of the buds is often lethal to the seedling (Glerum 1985). These are qualitative assessments (Johnson and Gagnon 1988) which are used to gauge when to lift and plant bareroot and container seedlings (Burdett and Simpson 1984).

A technique widely used in Ontario (Colombo and Glerum 1984; Colombo *et al.* 1984) is based on electrical conductivity. The technique assumes that frost-injured plant cells exhibit membrane disruption, which allows cytoplasmic material to leak into the xylem (Ritchie 1984). By comparing the electrical conductivity of xylem diffusate from untreated, frost-damaged and frost-killed seedlings, one can rank the degree of injury (Colombo *et al.* 1984; Ritchie 1984; Colombo and Hickie 1987). This is a quantitative assessment which is also used to gauge lifting dates.

Woody plants produce the gases ethylene, ethane, acetaldehyde and ethanol in response to stresses such as air pollutants (NO_x), water deficits, freezing and O_3 (Kimmerer and Kozlowski 1982). Of the four primary gases, it appears that ethanol and acetaldehyde are the best for screening stress resistance or quality of plant tissue (Kimmerer 1987; Kimmerer and MacDonald 1987; Kimmerer and Stringer 1988; MacDonald *et al.* 1989). The SIVE (stress-induced volatile emission) test being developed in MacMillan Bloedel's laboratory is based on gas emissions by woody plants (Drakeford and Hawkins 1989) and has the potential to complement or supersede the existing frost tests because of its speed (2–3 hours) and quantitative basis. Johnson and Gagnon (1988) have found ethane production to be as sensitive as electrolyte leakage from needles for predicting frost resistance in loblolly pine (*Pinus taeda*).

This report describes a series of stress resistance assessments on hybrid spruce (*Picea sitchensis* X *P. glauca* (Bong.) Carr., a naturally occurring hybrid), compares SIVE testing to conventional tests, outlines seasonal nursery baseline acetaldehyde and ethanol production in Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) seedlings, and describes Douglas-fir seasonal response to low temperature stresses as assessed by SIVE and conventional methodology.

2 MATERIALS AND METHODS

2.1 Experiment 1

The responses of a naturally occurring hybrid of interior (*Picea glauca*) and Sitka (*P. sitchensis*) spruce (Sxs) to low temperature stresses were observed before and after removal (planting) from freezer/cold storage. The stock was stored at -2°C from November 2, 1988, to April 1, 1989, and held at about +2°C until planting on May 23, 1989.

The Sxs registered Seedlot 3958 was raised at Red Rock Research Station, near Prince George, British Columbia. It was grown under four photoperiods ([P] nominal 13-, 15-, 17- and 19-hour days, denoted P1 to P4, respectively) each applied for three durations ([D] 2, 4 or 6 weeks application, respectively called D1 to D3) during the 1988 crop year (Hawkins and Draper 1988, 1991). Stock from all 12 photoperiod × duration (P×D) combinations was placed into freeze storage on November 2, 1988.

Conditioning of the stock (thawing slowly) started at the beginning of April 1989 (see Hawkins and Draper 1991 for details). Thawed stock was shipped by refrigerated van from Prince George in early May 1989. Stock was held at +2°C in the coolers at the MacMillan Bloedel, Angus P. MacBean Nursery, near Nanaimo, before planting.

2.1.1 Pre-planting assessments

Seedlings from all photoperiod treatments were brought from the cooler to the laboratory where they were then exposed to three temperatures: a positive control (room temperature, ≈23°C), -7°C and -17°C. Fifteen seedlings from each P×D combination were subjected to each test temperature. Two seedlings were used for SIVE, three for electrolyte leakage (conductivity), five for visual damage assessments, and five for survival (post-freezing planting). This was replicated twice, giving 30 seedlings per P×D combination at each temperature. All nursery P×D combinations were tested before planting (12 nursery treatments by 3 temperatures).

Freezing

Seedlings were placed in vermiculite insulated 313B styroblocks (from Beaver Plastics, Edmonton, Alberta) which were put in a Forma Scientific Bio-Freezer (Marietta, Ohio) and held at +3±0.5°C for 1 hour. The freezer temperature was then lowered at $6°C \cdot h^{-1}$ with an Omron E5T temperature controller (Omron Tateisi Electronics Co., Japan), until the desired test temperature (-7°C or -17°C) was reached. This temperature was held (through soaking) for 1 hour. Longer soaks may increase tissue damage (Ritchie 1984; Glerum 1985). The temperature was then ramped up to +3°C at the rate of $12°C \cdot h^{-1}$. Seedlings in styroblocks were removed from the freezer after a 1-hour soak at +3°C and allowed to sit at laboratory temperatures (≈23°C) and light intensities (≈50 $\mu mols \cdot m^{2} \cdot s^{-1}$). Sampling for SIVE and electrical conductivity began 5 and 15 minutes, respectively, after the stock was removed from the freezer. The entire procedure was repeated for the second negative temperature (-17°C).

SIVE sampling

SIVE sampling was conducted as previously described (Drakeford and Hawkins 1989), but with the following modifications. Shoots were placed in 57 mL glass test tubes which had been flushed with hydrocarbon free air. The tube was sealed, flushed for 5 seconds with hydrocarbon free air and allowed to incubate at room temperature (23±2°C) for 2 hours. After incubation, a 50 mL sample of headspace gas was withdrawn from the sealed test tube (see Drakeford and Hawkins [1989] for obtaining headspace gas).

When the gases of interest were ethane, acetaldehyde and ethanol, the 50-mL sample was injected into the coldtrap and processed as previously described (Drakeford and Hawkins 1989). A modified program was used when the gases of interest were only acetaldehyde and ethanol (Appendix 1). Coldtrapping was done in an ice bath (±0°C) rather than in a $CaCl_2$ bath (±-21°C) with this program. The modified program allowed twice as many samples to be processed in a day.

Electrical conductivity

The electrical conductivity or electrolyte leakage test to assess low temperature-induced shoot damage was modified from Colombo and Hickie (1987). Seedling shoots from each P×D combination tested at each of the three temperatures were cut into 7.5-cm segments, weighed and placed in a 150-mL plastic jar. Entire shoots were used because this was the basis of SIVE and foliage browning

assessments. The jar was filled to the brim with distilled water and capped. Initial electrical conductivity (EC) measurements were made on all samples after 24 hours in the distilled water at room temperature, with a YSI Model 32 Conductance Meter (Y.S.I. Corporation, Yellow Springs, Ohio). Measured EC samples were returned to their respective jars. The capped jars were placed in a forced-air oven at 90±1°C and held at this temperature for 2 hours to kill the tissue. A killed EC sample was determined after the sample had returned to room temperature, 24 hours after the oven kill. Standardized electrical conductivity values (EC_s) were calculated by dividing both the 24-hour and the killed EC values by the tissue fresh weight. An electrical conductivity damage ratio (Flint *et al.* 1967) was calculated for each sample:

$$\text{EC damage ratio} = \text{ECD} = \frac{\text{24-hour } EC_s}{EC_s \text{ kill}}$$

Visual assessments and survival

Two hours after removal from the freezer, the remaining seedlings were divided into two groups, each of which was placed in a styroblock and well watered.

One styroblock with seedlings was placed under full spectrum fluorescent g,ow lights (≈150 $\mu mols \cdot m^{2} \cdot s^{-1}$) in the laboratory to be assessed for visual damage. Blocks were watered to saturation every second day. Foliage, cambium and buds were scored separately for damage after 8 days in this environment (Ritchie 1984; Glerum 1985; Colombo and Glerum 1984). Foliage was scored as the percentage of needles injured on each seedling. Buds and cambium were assessed as being either healthy (0) or damaged (1).

The other styroblock and seedlings were returned, the day after removal from the freezer, to the A.P. MacBean Nursery open compound, placed under irrigation and followed for survival. Survival surveys were done fortnightly until mid-July and then monthly. Trees were removed from the styroblocks as they died. Only end-of-season, October 1989, survival is reported because of correlations done between end-of-season survival and test-day ethanol production and foliage browning.

2.1.2 Post-planting assessments

On May 23, 1989, 20 trees from each of the 12 P×D treatments were removed from cold storage and placed in 313B styroblocks under irrigation in the open compound at the A.P. MacBean Nursery. Baseline survival was determined from these seedlings. About 180 trees from each of the 12 P×D treatments were also "planted" in 313B styroblocks at this time for later post-planting, low temperature, stress-resistance assessments.

Due to logistical constraints, four P×D treatment combinations were the maximum that could be tested on a given day. From the seedlings "planted" on May 23, the four photoperiods of a given duration (e.g., photoperiods 1–4 [P1–P4] all of duration 2 [D2]) were brought to the laboratory and subjected to three levels of temperature to determine their post-planting frost hardiness.

As in Section 2.1.1, each temperature and P×D treatment was replicated, resulting in 30 seedlings per test temperature of each photoperiod of a given duration. Initially (7, 17 and 23 days after planting), all photoperiods from each duration were tested at control (≈23°C), -7°C and -17°C temperatures, identical to the pre-plant test. Low temperature damage increased as time since planting increased. Therefore, temperatures were raised to -3°C and -7°C, except for duration 3 (D3, P1–P4) which was tested at -3°C and -9°C. (The change to -3°C and -7°C was made so there would be a common negative temperature for all test times.)

Sub-samples of stock from each temperature and P×D combination were assessed for damage by SIVE, electrical conductivity and visual means, and for survival, with stock being returned in a styroblock to the irrigated nursery compound (as described in Section 2.1.1).

2.1.3 Statistical analysis

This experiment was designed as a randomized factorial. A mixed model ANOVA (BMDP 8V) was used for pre-planting analysis (BMDP 1988). Duration (D), photoperiod (P) and temperature (T) were fixed factors; replicate (R) was random (Table 1).

Post-planting analyses were done on replicated, three-temperature, four-photoperiod, one-duration samples (i.e., the duration tested on a given date after planting [e.g., D1, P1–P4]). A mixed model ANOVA (BMDP 7D) with photoperiod and temperature fixed and replicate random (Table 2) was used for this analysis (BMDP 1988).

Proportional or percentage data was transformed by arcsin (BMDP 1988) before the ANOVA was run. Mean values reported in tables are of non-transformed data.

Linear regressions were performed on SYSTAT® as described by Wilkinson (1988) on end-of-season survival versus ethanol production and foliage browning. The best fit regressions are reported.

TABLE 1. ANOVA model used for assessment of pre-planting experimental variables. Duration, photoperiod and temperature are fixed and replication is random.

Source	Df	Error term	Expected mean square
Duration	2	$\Sigma_{Q(ijk)}$	$\sigma_{\Sigma}^2 + 24\sigma^2_{D}$
Photoperiod	3	$\Sigma_{Q(ijk)}$	$\sigma_{\Sigma}^2 + 18\ \sigma^2_{P}$
Temperature	2	$\Sigma_{Q(ijk)}$	$\sigma_{\Sigma}^2 + 24\ \sigma^2_{T}$
DP	6	$\Sigma_{Q(ijk)}$	$\sigma_{\Sigma}^2 + 6\ \sigma^2_{DP}$
DT	4	$\Sigma_{Q(ijk)}$	$\sigma_{\Sigma}^2 + 8\ \sigma^2_{DT}$
PT	6	$\Sigma_{Q(ijk)}$	$\sigma_{\Sigma}^2 + 6\ \sigma^2_{PT}$
DPT	12	$\Sigma_{Q(ijk)}$	$\sigma_{\Sigma}^2 + 2\ \sigma^2_{DPT}$
$\Sigma_{Q(ijk)}$	36		
Total	71		

TABLE 2. ANOVA model used for assessing post-planting experimental variables. Photoperiod and temperature are fixed and replication is random.

Source	Df	Error term	Expected mean square
Photoperiod	3	$\Sigma_{Q(ij)}$	$\sigma_{\Sigma}^2 + 6\ \sigma^2_{P}$
Temperature	2	$\Sigma_{Q(ij)}$	$\sigma_{\Sigma}^2 + 8\ \sigma^2_{T}$
PT	6	$\Sigma_{Q(ij)}$	$\sigma_{\Sigma}^2 + 2\ \sigma^2_{PT}$
$\Sigma_{Q(ij)}$	12		
Total	23		

2.2 Experiment 2

2.2.1 Douglas-fir seasonal baseline

Douglas-fir registered Seedlot 510 was sown into 66, 313A styroblocks on April 3, 1989, at the A.P. MacBean Nursery. Stock was grown under a standard cultural regime until July 18, when operational drought stressing to control seedling height (Matthews 1981, 1983) was applied to 33 of the blocks for 4 weeks. Blocks were rewatered when total block weight loss was at least 3000 g (pre-dawn, plant moisture status ≈-8 to -10 bars, E.A. Maher, Superintendent, A.P. MacBean Nursery, pers. comm., July 1989). After drought treatments, all stock was again grown under a common standard cultural regime and watered when block weight loss was about 2000 g.

Beginning on June 29, before initiation of drought treatments, Douglas-fir stock was brought to the laboratory to ascertain SIVE baseline gas (acetaldehyde and ethanol) emissions on five to eight seedlings per future designated nursery treatment. Baseline electrical conductivity was also calculated on an additional three to six seedlings per treatment. Both techniques were carried out as previously described (Section 2.1.1).

2.3 Experiment 3

2.3.1 Douglas-fir response to low temperature

Douglas-fir seedlings described in Section 2.2.1 were also used to determine seasonal seedling response to low temperature exposure.

Starting in early August, stock was transported from the nursery to the laboratory and subjected to five levels of temperature that were progressively lowered during the season as seedling frost resistance increased. Seedlings were placed in partial styroblocks in a freezer and held at +3°C for at least 1 hour (soak). After the soak, the freezer temperature was lowered at the rate of $6°C \cdot h^{-1}$ (ramp) until the desired temperature was reached. This temperature was held for 15 minutes, during which time the sample was removed and placed in an ice chest at 0°C (see Keates' [1990] discussion of Tinus' freezer methodology). After the 15-minute soak, the freezer ramped down to the next temperature and the next sample was removed during the soak phase. This was repeated until four negative temperature samples had been taken. See Sections 3.2 and 3.4 for rationale behind freezing protocol changes.

Five and 15 minutes after a seedling temperature sample was placed in the 0°C ice chest, subsamples were taken for SIVE (three to six seedlings per nursery treatment per temperature) and electrical conductivity (two to four seedlings per nursery treatment per temperature), respectively. Volatile gas production and electrical conductivity damage ratio were calculated for each test temperature on each test date. After 1 hour in the ice chest at 0°C, the remaining seedlings (4–10 per nursery treatment per temperature) were removed, covered with black plastic and placed on a bench in the laboratory to allow the shoots to reach laboratory temperatures. The plastic was removed after 1 hour and the seedlings were well watered, and placed under full spectrum fluorescent grow lights (≈150 $\mu mols \cdot m^{2} \cdot s^{-1}$) in low humidity laboratory air for 1 week. Seedlings were watered to saturation every second day. Visual damage assessments were then done on foliage, buds and cambia.

Linear regressions were done between foliage browning (FB) and electrical conductivity damage ratio, FB and ethanol, FB and acetaldehyde concentrations and electrical conductivity damage and ethanol using SYSTAT® (Wilkinson 1988) when winter frost hardiness was attained.

3 RESULTS AND DISCUSSION

3.1 Experiment 1: Pre-planting Assessments

Following storage, both ethanol and acetaldehyde production were significantly increased by exposure to low temperatures (Table 3; Appendix 2). Electrical conductivity was significantly increased at increased durations (D), and at decreased photoperiods (P) and temperatures (T), and there was a D×T interaction

(Table 3; Appendix 2). No change occurred in standardized electrical conductivity (EC_s) between durations 2 and 3 at temperatures 1 and 2, but did between D2 and D3 for T3 (-17°C), hence the interaction (Appendix 3).

TABLE 3. Significance (significant $\alpha = 0.05$, *; non-significant, ns) of treatments on ethanol (EtOH) and acetaldehyde (Acet) production, standardized electrical conductivity (EC_s), foliage browning (FB), bud damage (BD), cambium damage (CD), and end-of season survival (Surv) in Sxs removed from cold storage and tested

Source	EtOH	Acet.	EC_s	FB	BD	CD	Surv.
Duration	ns	ns	*	*	ns	ns	ns
Photoperiod	ns	ns	*	*	*	*	*
Temperature	*	*	*	*	*	*	*
DP	ns	ns	ns	ns	*	*	ns
DT	ns	ns	*	*	ns	*	ns
PT	ns	ns	ns	*	ns	*	*
DPT	ns	ns	ns	ns	*	*	ns

Foliar browning (FB) increased with increased duration (D) and photoperiod (P), and with decreased temperature (T). As well, D×T and P×T interactions occurred (Table 3; Appendix 2). Between durations 2 and 3, FB decreased at temperature 2, while it was identical for D2 and D3 at T1 and T3. At T3, FB increased between photoperiods 2 and 3, but did not change between P2 and P3 for T1 and T2 (Appendix 3).

Bud damage (BD) increased with decreased photoperiod and temperature, and D×P and D×P×T interactions occurred (Table 3; Appendix 2). It increased with increased D for P1, but decreased or remained unchanged at photoperiods 2, 3 and 4. Hence, the first-order interaction was recorded (Appendix 3).

Cambium damage (CD) increased with increased photoperiod and temperature, and there were first- and second-order interactions (Table 3; Appendix 2). At P3, CD was constant for all durations, but increased with increased duration at P1, decreased with increased duration at P2, and did none of the above at P4. Cambium damage was the same at temperatures 1 and 2 for all durations and it increased with increased duration at T3. The CD pattern of interaction for P×T was the same as for D×T (Appendix 3).

Survival decreased with decreased photoperiod and temperature, and a P×T interaction occurred (Table 3; Appendix 2). Survival decreased between P3 and P4 at T3, while it increased between P3 and P4 at T1 and T2 (Appendix 3). An electrical conductivity damage (ECD) ratio was not calculated for the D1 sample, and was therefore not analyzed in the ANOVA model. However, ECD ratio did increase at decreased temperatures for stock from durations 2 and 3.

Test temperature had the greatest effect on response variables, followed by nursery photoperiod and application duration (Table 3). This is probably consistent with the severity of the factors analyzed by the ANOVA model. The greater the potential physiological effect of the factor, the greater are response variables affected by it.

For the variables that temperature significantly affected (Table 3), there was little change between control temperature and -7°C, but a large difference between the values at -7 and -17°C (Table 4). These data suggest that the SIVE technique is consistent with, and could replace, standard visual and conductivity assessments of freezing stress damage provided the costs per sample were similar. The data also indicate that this stock had sufficient stress resistance to withstand a -17°C stress event and still had, on average, less than 35% foliage browning after more than 6 months of freezer/cold storage, and a survival rate greater than 50%. Low visual (temperature-induced) damage scores are associated with the high end of season rates of survival.

TABLE 4. The effect of temperature on ethanol (EtOH) and acetaldehyde (Acet) production, standardized electrical conductivity (EC_s), foliage browning (FB), bud damage (BD), cambium damage (CD), and end-of-season survival (Surv) in Sxs removed from cold storage and tested. Values are pooled means.

Temperature	EtOH nmols-g^{-1}	Acet. nmols-g^{-1}	EC_s µmhos-cm^{-1}	FB %	BD %	CD %	Surv. %
1, ≈23°C	0.036	0.000	16.00	0.0	0.0	0.0	89.2
2, -7°C	0.444	0.395	18.17	6.5	14.9	0.4	83.3
3, -17°C	5.745	4.660	30.33	34.6	76.0	22.3	56.7

The effect of photoperiod on the significantly affected response variables (Table 3) was inconsistent (Table 5). Standardized electrical conductivity decreased and foliar browning and cambium damage increased with increasing photoperiod, while bud damage was greatest and survival poorest under the extreme photoperiod treatments. The differences in responses to nursery photoperiod among visual damage assessment variables (Table 5) indicates two points: 1) the effect of nursery cultural treatment is not the same on all plant parts and there are inherent inconsistencies associated with a qualitative assessment; and 2) a physiologically based test for each plant part would not only alleviate some of the variation, but would make the assessment more quantitative.

Standardized electrical conductivity (EC_s) increased with increased application duration, while foliar browning increased and plateaued (Table 6). These data indicate that nursery treatment effects can be observed after more than 6 months of freezer/cold storage. These effects are also evident on seedling survival after storage, a stress treatment and a growing season (Tables 4 and 5). Interestingly, EC_s indicates more significant treatment differences than does survival, while volatile (ethanol and acetaldehyde) production indicates fewer treatment differences than survival. The increased sensitivity of EC_s compared to that of SIVE likely indicates the former's longer period of test development and application. It is probably better to be assessing stock with a conservative test (SIVE) than a liberal test (EC_s), because of operational logistical realities.

As neither photoperiod nor duration was significant for ethanol production (Table 3), a regression was done with replicate means of ethanol production and end-of-season seedling survival (Figure 1) for all P×D×T treatments (n = 72). The resultant equation was:

[1] Survival = -0.034(ethanol) + 0.856

The correlation coefficient was -0.584, which is significant at $P > 0.999$. The relationship between survival and foliage browning (Figure 2) was also determined:

[2] Survival = -1.005(FB) + 0.927

The correlation coefficient was -0.772, which is significant at $P > 0.999$.

TABLE 5. The effect of photoperiod on standardized electrical conductivity (EC_s), foliage browning (FB), bud damage (BD), cambium damage (CD), and end-of-season survival (Surv) in Sxs removed from cold storage and tested. Values are pooled means.

Photoperiod	EC_s µmhos*cm^{-1}	FB %	BD %	CD %	Surv. %
1, 13 h	26.26	11.7	36.3	5.6	62.8
2, 15 h	21.02	10.3	29.2	2.7	82.2
3, 17 h	18.58	13.3	23.4	6.6	84.4
4, 19 h (ambient)	20.13	19.4	32.2	15.4	76.1

TABLE 6. The effect of photoperiod application duration on standardized electrical conductivity (EC_s) and foliage browning (FB) in Sxs. Values are pooled means.

Duration	EC_s $\mu mhos\text{-}cm^{-1}$	FB %
1, 2 weeks	16.22	8.5
2, 4 weeks	22.76	17.5
3, 6 weeks	25.53	15.0

Therefore, for Sxs spruce removed directly from storage, subjected to low temperature stress events and then outplanted, ethanol production and foliage browning both correlated to end-of-season survival. Although foliage browning showed a better correlation to survival, ethanol production has the advantage of delivering quantitative results on the same day as the stress test rather than a week after the test.

3.2 Experiment 1: Post-planting Assessments

After planting, for the response variables examined, temperature was the only model component to have a significant response effect on all variables examined (Table 7), except for standardized electrical conductivity on day 9, duration 3. For the first 23 days after planting, the test temperatures used were control (≈23°C), -7°C and -17°C. Once buds had broken, warmer test temperatures were used and the significance of model components on response variables increased (Table 7, days 28, 30 and 35). Again, temperature had the most frequent, significant impact on assessed variables and there were few P×T interactions. Buds were not assessed after day 23 because stock from all 12 treatments was rapidly elongating (flushing).

Generally, volatile production, standardized electrical conductivity, electrical conductivity damage ratio, and foliar, bud and cambium damage increased with decreased temperature, while survival decreased with exposure to decreased temperature (Table 8). Survival was usually over 70% for seedlings subjected to -7°C and outplanted, regardless of whether they were flushing or not at the time of exposure.

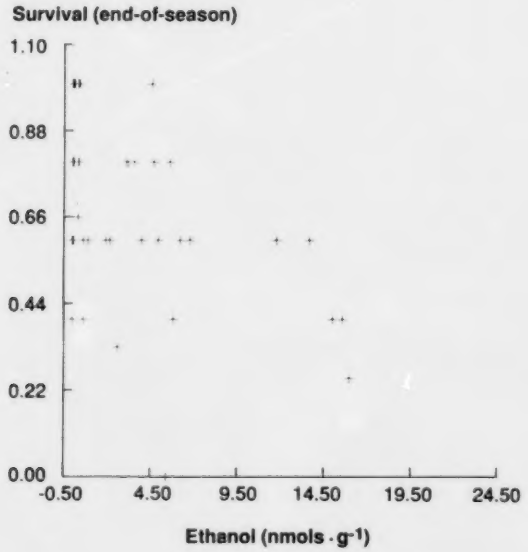

FIGURE 1. End-of-season mean survival versus mean ethanol (EtOH) production for all P×D×T nursery treatments of Sxs spruce exposed to temperature stresses directly on removal from storage, n = 72.

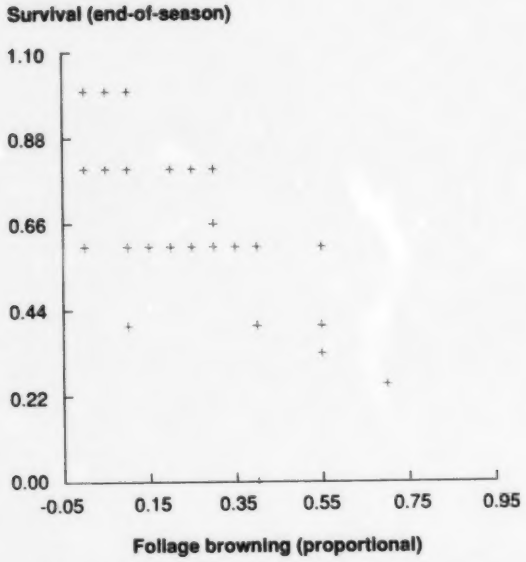

FIGURE 2. End-of-season mean survival versus mean foliage browning (FB) for all P×D×T nursery treatments of Sxs spruce exposed to temperature stresses directly on removal from cold storage, n = 72.

TABLE 7. Significance (significant $\alpha = 0.05$, *; non-significant, ns) of treatments on ethanol (EtOH) and acetaldehyde (Acet) production, standardized electrical conductivity (EC_s), EC damage ratio (ECD), foliage browning (FB), bud damage (BD), cambium damage (CD), and end-of-season survival (Surv) in Sxs exposed to low temperature stresses at a various number of days after planting. A different duration was tested on each date. Each day or treatment duration was analyzed as a separate ANOVA.

Source	Acet.	EtOH	EC_s	ECD	FB	BD	CD	Surv.
9 days, D3								
P	ns	ns	*	ns	ns	ns	ns	ns
T	*	*	*	*	*	*	*	*
PT	ns	ns	ns	ns	ns	ns	ns	ns
17 days, D1								
P	ns	ns	ns	ns	ns	ns	ns	ns
T	*	*	*	*	*	*	*	*
PT	ns	ns	ns	ns	ns	ns	ns	ns
23 days, D2								
P	ns	ns	ns	ns	ns	ns	ns	ns
T	*	*	*	*	*	*	*	*
PT	ns	ns	ns	ns	ns	ns	ns	ns
			Test temperature change					
28 days, D3								
P	ns	ns	ns	ns	*	-	ns	ns
T	*	*	*	*	*	-	*	*
PT	ns	ns	ns	ns	*	-	ns	ns
30 days, D1								
P	ns	ns	*	*	*	-	ns	*
T	ns	*	*	*	*	-	ns	ns
PT	ns	ns	ns	ns	*	-	ns	ns
35 days, D2								
P	*	*	ns	*	*	-	ns	*
T	*	*	*	*	*	-	ns	*
PT	*	ns	ns	*	*	-	ns	ns

TABLE 8. The effect of temperature (Temp) on ethanol (EtOH) and acetaldehyde (Acet) production, standardized electrical conductivity (EC_s), EC damage ratio (ECD), foliage browning (FB), bud damage (BD), cambium damage (CD), and end-of-season survival (Surv) in Sxs exposed to low temperature stresses at a various number of days after planting. A different duration was tested on each date. Each day or treatment duration was analyzed as a separate ANOVA. Values are pooled means.

Temp.	Acet. nmols-g^{-1}	EtOH nmols-g^{-1}	EC_s µmhos-cm^{-1}	ECD %	FB %	BD %	CD %	Surv. %
9 days, D3								
≈23°C	0.000	0.003	15.37	16.4	0	0	0	88
-7°C	2.045	2.680	17.93	19.0	15	46	0	93
-17°C	6.700	10.180	25.26	24.0	63	100	52	58
17 days, D1								
≈23°C	0.018	0.394	6.59	8.4	0	0	0	95
-7°C	0.255	0.487	8.81	11.2	18	9	4	80
-17°C	9.004	4.685	15.93	17.5	65	100	87	0
23 days, D2								
≈23°C	0.123	0.213	5.18	7.1	0	0	0	97
-7°C	0.421	0.905	6.00	8.1	29	39	4	60
-17°C	2.255	1.814	12.79	15.2	94	100	88	0
			Test temperature change					
28 days, D3								
≈23°C	0.202	0.427	4.95	6.5	1	—	0	93
-3°C	0.132	0.310	4.33	5.5	6	—	0	88
-9°C	1.115	2.014	12.05	15.2	65	—	18	23
30 days, D1								
≈23°C	0.088	0.120	4.83	6.7	1	—	0	86
-3°C	0.026	0.151	4.43	6.0	4	—	0	89
-7°C	0.474	0.928	6.65	9.7	42	—	0	72
35 days, D2								
≈23°C	0.123	0.205	4.41	6.7	2	—	0	88
-3°C	0.026	0.180	3.76	5.8	8	—	0	83
-7°C	0.493	0.515	8.24	12.7	22	—	0	68

This is a curious result, as -5°C frosts can devastate a plantation whose shoots are elongating in the spring. Obviously, the simulation of a low temperature stress event is a poor representation of the plantation situation. Perhaps, as most spring frosts occur when soils are cold and the events are followed by warm days with high irradiance, it is the post-freezing, light, temperature and drought stress interactions that are missing from present test routines.

As the effect of photoperiod was generally not significant after planting (Table 7), regressions were done with replicate means from each post-planting test day, days 9, 17, 23, 28, 30 and 35 (e.g., D1; P1-P4; T1-T3; n = 24). Survival was significantly correlated to ethanol production on days 17, 23, 28 and 35 (Table 9). The lack of correlation on days 9 and 30 was due to insufficient ethanol production at the test temperatures used. It is not known whether this was a function of the stock on the test day or to pre-testing protocol. Survival correlated to foliage browning on all 6 test days (Table 9). The correlation was stronger for FB than for ethanol production. It is anticipated that once better pre-test protocols and baseline gas production definitions are achieved, the technique will start to approach its potential of complementing some of the existing tests. These objectives will be met because SIVE is a rapid, reliable and quantitative assessment of seedling vigor.

3.3 Experiment 2: Seasonal Baseline in Douglas-fir

Seasonal acetaldehyde production was variable, ranging from 0 to 1.5 nmols-g^{-1} (Figure 3). There was no marked effect on acetaldehyde production during the nursery drought treatment, July 18 through August 15, as both control and drought-treated seedlings changed similarly. Although differences among nursery

treatments were large on some dates, none was significant except on January 16. However, at the time of lifting and storage, there was no nursery treatment difference.

Seasonal ethanol production was variable and ranged from about 0.3 to 1.7 nmols·g^{-1} (Figure 4). There was no significant difference in ethanol production during the 4 weeks of nursery treatment application, but drought-treated seedlings produced significantly more ethanol 3 weeks after the end of the drought treatment, September 6. This may indicate that the stresses are physiologically more significant after drought treatment when environmental factors favor maximum productivity.

TABLE 9. Regression equation, correlation coefficient (r), and level of significance (P[F]; P[F]<0.050 is significant) for end-of-season survival as a function of ethanol (ET) production and foliage browning (FB) in Sxs after a post-planting, low temperature stress test

Days planted	Equation	r	P(F)
9	SURV= -0.015(ET) + 0.832	-0.22	0.303
17	SURV= -0.139(ET) + 0.842	-0.78	0.000
23	SURV= -0.324(ET) + 0.839	-0.68	0.000
28	SURV= -0.239(ET) + 0.894	-0.59	0.002
30	SURV= 0.010(ET) + 0.811	0.02	0.928
35	SURV= -0.472(ET) + 0.936	-0.52	0.010
9	SURV= -0.550(FB) + 0.925	-0.56	0.005
17	SURV= -1.451(FB) + 0.987	-0.93	0.000
23	SURV= -1.005(FB) + 0.936	-0.91	0.000
28	SURV= -0.411(FB) + 0.894	-0.84	0.000
30	SURV= -0.592(FB) + 0.907	-0.44	0.000
35	SURV= -0.690(FB) + 0.913	-0.51	0.012

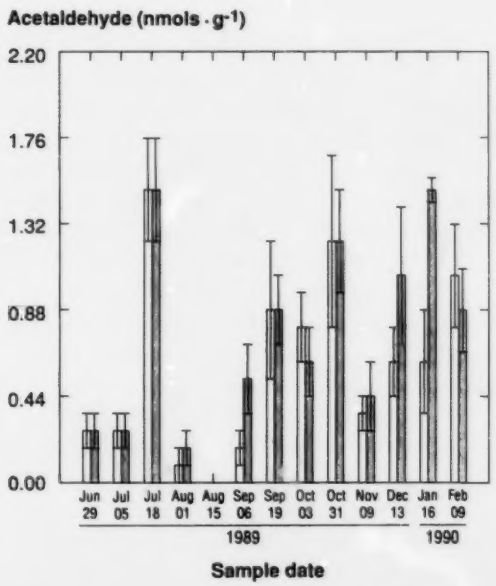

FIGURE 3. Seasonal mean (±SE of mean) acetaldehyde production (nmols·g^{-1}) of control (clear) and drought-treated (slash) Douglas-fir grown at the A.P. MacBean Nursery in 1989/1990.

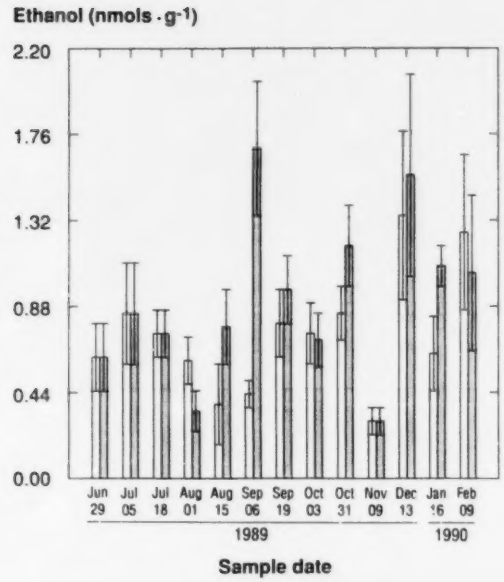

FIGURE 4. Seasonal mean (±SE of mean) ethanol (EtOH) production (nmols•g^{-1}) of control (clear) and drought-treated (slash) Douglas-fir grown in 1989/1990.

Generally, after drought treatment, the control seedlings had lower levels of ethanol production. Drought-treated seedlings produced significantly more ethanol in mid-January. At lifting and storage, there was no difference between treatments, and no seasonal pattern for ethanol production in Douglas-fir emerged. There was, however, a trend for increased ethanol production with seedling maturation (Figure 4). Non-treated or control seedling ethanol production did not exceed 0.85 nmols•g^{-1} up to mid-November. This information can be used to establish a postulated critical ethanol threshold of 1.0 nmols•g^{-1} to indicate the onset of seedling stress during this period. Ethanol production was greater during the winter, and the critical threshold for this period should be 1.75 nmols•g^{-1} or greater.

Seedling ethanol stress threshold establishment requires more baseline information, as there have been no replications in time or space.

The ratio of acetaldehyde to ethanol (proven useful in diagnosing stress responses during preliminary studies; DeYoe 1987, 1988 and Hawkins 1989, unpublished FRDA interim file reports) ranged from 0 to 2.25 (Figure 5). The ratio was low before (except for July 18, no explanation for rise), during, and just after drought treatment for both treatment groups, and then it increased. After treatment, the drought-treated seedlings usually had the greatest ratio, possibly indicating a greater level of cumulative seasonal stress. There was no difference between treatments at lifting and storage, and a seasonal pattern did not emerge.

The first seasonal volatile gas baseline indicates that SIVE data are variable, but that seedling response to nursery drought treatments can be detected. This is encouraging, but indicates that baseline protocols are going to require better definition if SIVE technology is to be used as a nursery culture monitor. Samples will have to be taken at 7- to 10-day intervals to establish the baseline. It also appears that ethanol alone is the best variable to be incorporated into a stand-alone screening test. This is supported by work from Kimmerer's laboratory (Kimmerer and MacDonald 1987; Kimmerer and Stringer 1988; MacDonald *et al.* 1989).

Ethanol is likely the best gas because it is continually produced in woody plants both under aerobic and anaerobic conditions (Kimmerer 1987; Kimmerer and MacDonald 1987; Kimmerer and Stringer 1988). Ethane production was monitored, too, but is not reported because it did not distinguish between control and drought-treated stock. This indicates there was no membrane damage associated with the treatments at the levels applied. However, ethane can (Johnson and Gagnon 1988) and will play a crucial role in sublethal being distinguished from lethal stresses in laboratory assessments after initial seedling screening protocols have

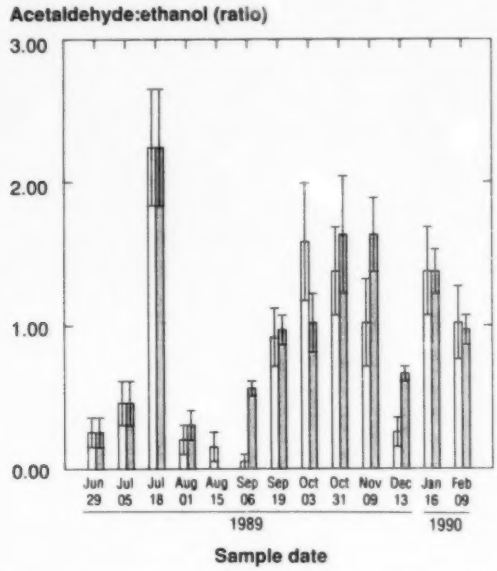

FIGURE 5. Seasonal mean (±SE of mean) acetaldehyde to ethanol (Acet:EtOH) ratio of control (clear) and drought-treated (slash) Douglas-fir grown in 1989/1990.

been completed. In recent experiments, high ethane concentrations indicated rapid and elevated seedling mortality, even though ethanol and acetaldehyde concentrations were not maximal for the experiment (C.D.B. Hawkins, unpublished data). This could be an indication of physiological differences between stress events and stress responses.

Seasonal standardized electrical conductivity (EC_s) generally declined with time and ranged from about 11 to 3 $\mu mhos \cdot cm^{-1}$ (Figure 6). Drought-treated stock had greater EC_s during and immediately after treatment; the control stock had greater values later in the growing season. No nursery treatment difference for EC_s occurred at lifting and storage. Shortly after the "fall drop" (October 13–31, Figure 6) in EC_s, bud and cambium frost tolerance increased markedly (Section 3.4). Perhaps EC_s also has the potential to be another cultural monitoring tool in the nursery, once enough knowledge is gained about seasonal EC_s patterns and correlations are made between it, seedling storability, and subsequent survival and growth.

Seasonal electrical conductivity damage (ECD) ratio ranged from 0.04 to 0.11 and was variable with time (Figure 7). During and after the drought treatment, ECD levels were lowest in control stock. As the season progressed, ECD levels were greatest in control stock, but there was no treatment difference at the time of lift and storage. The ECD ratio does not detect the fall drop indicated by standardized electrical conductivity (Figure 6). This reinforces the need for more work on the relationships between electrolyte leakage and other assessments to enhance the utility of the electrical conductivity technique.

Seasonal electrical conductivity data appear to indicate differences during and after nursery treatments, as does ethanol production, but acetaldehyde production does not. However, both SIVE and EC_s require considerably more operational baseline calibration. Perhaps in the nursery a viable seasonal crop-monitoring scheme can be developed with entire seedling shoots and SIVE ethanol screening and electrical conductivity measurements. Regardless of the method chosen, a rapid, reliable and predictive nursery crop monitoring scheme must be developed.

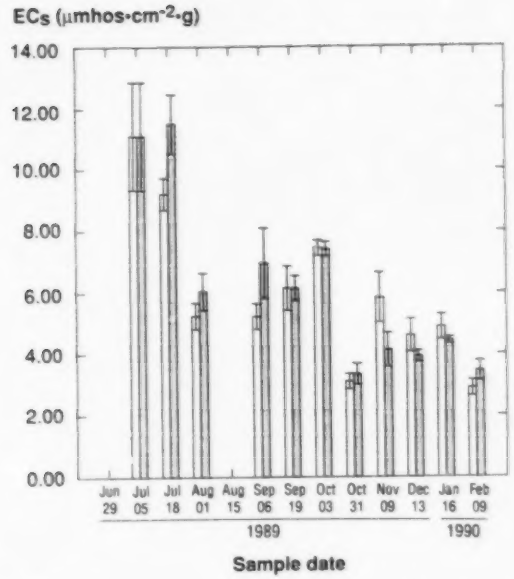

FIGURE 6. Seasonal change of mean (±SE of mean) standardized electrical conductivity (EC_s; μmhos·cm^{-2}·g) of control (clear) and drought-treated (slash) Douglas-fir grown in 1989/1990.

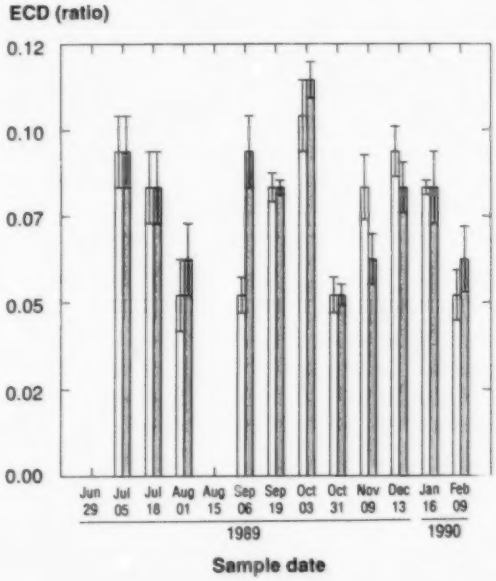

FIGURE 7. Seasonal mean (±SE of mean) electrical conductivity damage (ECD) ratio of control (clear) and drought-treated (slash) Douglas-fir grown at nursery in 1989/1990.

3.4 Experiment 3: Response to Low Temperature in Douglas-fir

A more "stressful" freeze treatment was used for these assessments because of the very high seedling resistance to low temperatures observed in Section 3.2. It is assumed this protocol results in greater stresses on the seedlings and more "realistic" post-exposure simulations of plant response to low temperature.

The season can be divided into four phases of frost tolerance as indicated by the production of ethanol and acetaldehyde, and visual damage assessments. In August and September, Phase I, during initiation of bud scales and filling of buds, exposure to lower temperatures resulted in unchanged or decreased ethanol production and increased acetaldehyde production (Figure 8). Electrical conductivity damage (ECD) ratio increased with decreased temperature and then decreased (Figure 9). The LT_{25} (temperature where 25% of the needles are damaged) for foliage, buds and cambia was about -6°C (Table 10). The decline in LT_{25} with exposure to lower temperatures usually indicates, for both ethanol and ECD, very high seedling mortality at the lowest test temperatures.

During late September and October, Phase II, both ethanol production and acetaldehyde production increased to higher levels than in Phase I with decreased temperature (Figure 10), though ethanol production tended to plateau. Electrical conductivity damage increased with decreased temperature for drought-treated stock but decreased at the lowest temperature in the controls (Figure 9). The LT_{25} of the foliage increased in controls and remained unchanged in drought-treated seedlings, while buds and cambia remained at about -6°C (Table 10). Generally during Phase II, nursery drought-treated stock had lower levels of damage, regardless of the variable being examined.

Ethanol production increased and acetaldehyde production decreased during Phase III (Figure 11), late October and early November, compared to that observed in the preceding phase (Figure 10). Foliar LT_{25} remained about -4°C while bud and cambium LT_{25} decreased to less than -13°C (Table 10). Electrical conductivity damage ratio again increased with decreased temperature and did not differ between nursery treatments (Figure 12). This phase corresponds to the "fall drop" in baseline EC_s observed in Section 3.3 (Figure 6).

From late November through to lifting in early February, Phase IV, maximum frost hardiness was achieved. Volatile gas production and electrical conductivity damage ratios were greatest in control stock (Figures 13 and 14), but there was little difference between nursery treatments for visual damage assess-

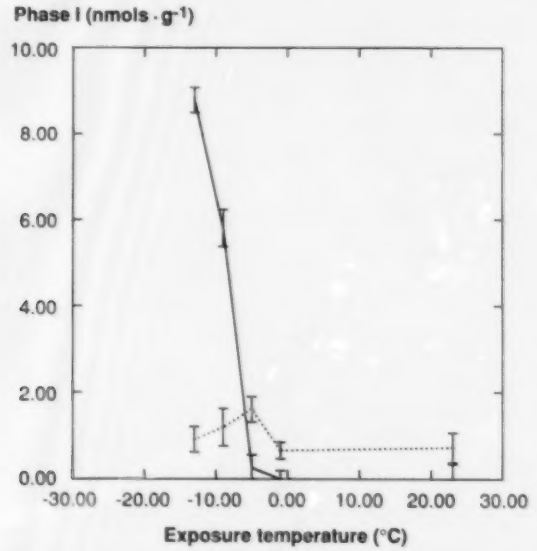

FIGURE 8. Phase I mean (±SE) ethanol (dotted line) and acetaldehyde (solid line) production in Douglas-fir seedlings exposed to the indicated temperatures.

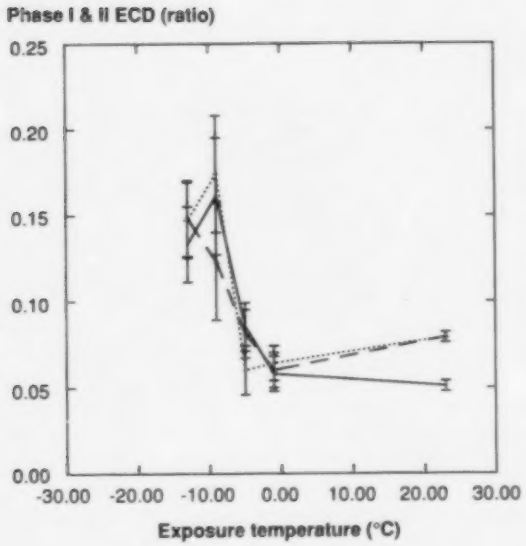

FIGURE 9. Mean (±SE) electrical conductivity (ECD) damage ratio for nursery control and drought-treated Douglas-fir seedlings exposed to the indicated temperatures during Phases I (solid line) and II (control [dotted line] and drought- [long-dashed line] treated).

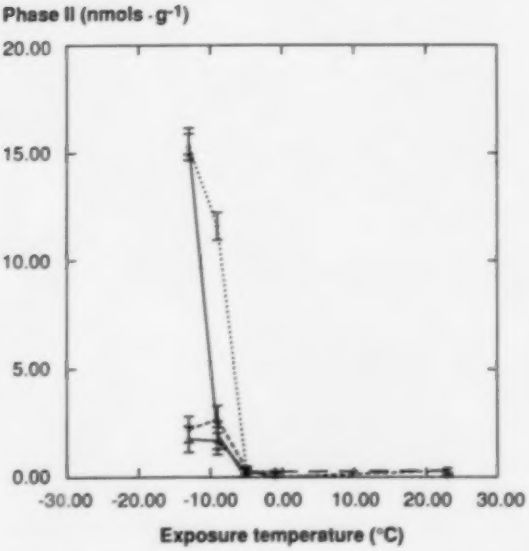

FIGURE 10. Phase II mean (±SE) acetaldehyde production (control [solid line], drought [dotted line]) and ethanol (control [long-dashed line], drought [short-dashed line]) in nursery control and drought-treated Douglas-fir seedlings exposed to the indicated temperatures.

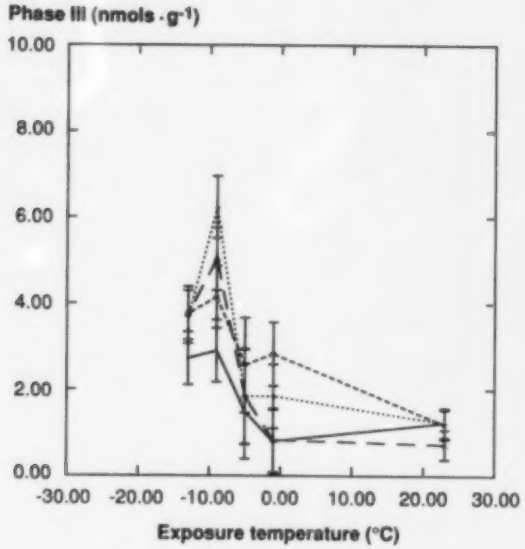

FIGURE 11. Phase III mean (±SE) acetaldehyde production (control [solid line], drought [dotted line]) and ethanol (control [long-dashed line], drought [short-dashed line]) in nursery control and drought-treated Douglas-fir seedlings exposed to the indicated temperatures.

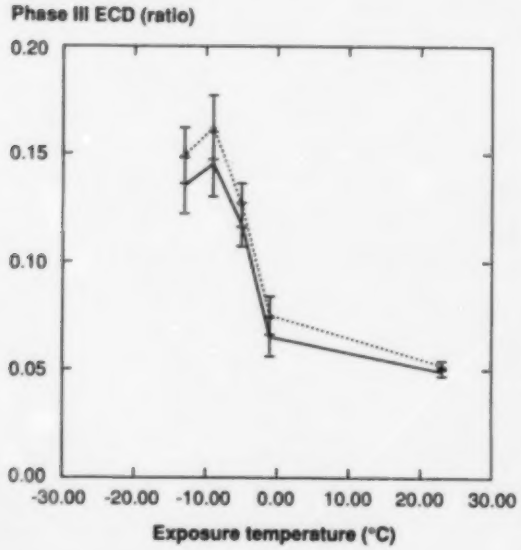

FIGURE 12. Mean (±SE) electrical conductivity damage ratio for nursery control (solid line) and drought-treated (dotted line) Douglas-fir seedlings exposed to the indicated temperatures during Phase III.

ments (Table 10). The stock's ability to withstand greater levels of low temperature stress is paralleled by greater levels of ethanol production. This could mean that high levels of ethanol production (>10 nmols•g^{-1}) indicate of high levels of external stress, and high levels of seedling stress resistance. This in turn could mean that the stock is ready for freeze/cold storage. Phase IV must be further investigated and better understood to refine the SIVE test.

Volatile and ECD levels during Phases II and III were generally not significantly different between nursery control and drought-treated seedlings (Figures 9–12). During Phase IV, volatile and ECD levels were significantly greater in control seedlings at the lowest test temperatures (Figures 13 and 14). It appears that, at low and moderate levels of temperature stress, nursery treatment had no effect on variable response. However, at greater levels of stress, nursery drought-treated seedlings were not affected as severely as control seedlings. This could indicate that nursery drought treatment preconditions the Douglas-fir seedling to withstand more severe abiotic stresses during Phase IV.

The concept of using threshold levels of ethanol production to indicate external stresses (Section 3.3) seemed to be valid during Phases I to IV. The suggested ethanol thresholds for Douglas-fir were 1.0 nmols•g^{-1} until mid-November and 1.75 nmols•g^{-1} from then until lifting. During Phases I and II, ethanol levels at or greater than 1.0 nmols•g^{-1} were associated with visual damage and seedling mortality (Figures 8 and 10, Table 10). Drought-treated stock did not exceed 0.6 mm^2•g^{-1} ethanol during Phase III until the LT_{25} was reached, whereas control stock exceeded the threshold after exposure to the first negative temperature (Figure 11, Table 10). This reflects nursery treatment differences observed during this phase for other assessment variables. During Phase IV, the ethanol threshold of 1.75 nmols•g^{-1} was not exceeded until visual damage was also observed (Figure 13, Table 10).

The idea of defining a seasonal stress threshold using ethanol production is appealing because it would allow screening to be done on "point in time" (snapshot) samples. Considerable utility will be added to the SIVE technique when stress response critical thresholds are clearly defined for the more important species.

Regression correlations have been done separately on control and drought-treated stock with mean values obtained during Phase IV. Ethanol, acetaldehyde and electrical conductivity damage correlated significantly with foliage browning in both treatments (Table 11). Ethanol production was also significantly correlated to ECD (Table 11). It is encouraging that ethanol production does correlate with the more established assessments of foliage browning and electrical conductivity. This supports the proposal that an ethanol screening procedure to assess seedling stress resistance can be established with the use of portable breath alcohol analyzers (MacDonald *et al.* 1989).

TABLE 10. LT_{25} (temperature which causes 25% damage to sample) of foliage (FOL), buds (BUD), and cambia (CAM) in nursery control and drought-treated Douglas-fir seedlings from August 1989 through February 1990. Not tested, nt.

	LT_{25} (°C)					
	Control			Drought		
Date	FOL	BUD	CAM	FOL	BUD	CAM
August 09	-6.3	nt	nt	-6.3	nt	nt
September 21	-6.2	-6.1	-6.1	-6.0	-6.0	-6.0
October 04	-3.7	-5.3	-6.0	-6.7	-5.8	-6.7
November 01	-2.7	-2.7	-10.0	-6.3	-6.0	-6.7
November 14	-4.5	<-13.0*	<-13.0	-3.5	<-13.0	<-13.0
December 12	-13.0	-16.5	-19.5	-18.0	-17.0	<-21.0
January 16	-8.6	-19.4	<-25.0	-9.0	-19.5	-22.4
February 08	-11.6	-16.5	<-21.0	-13.5	-16.5	<-21.0

* "<" indicates lowest temperature that stock was exposed to on date.

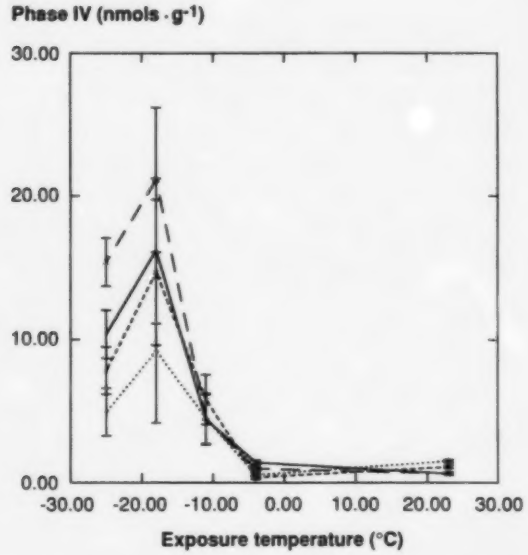

FIGURE 13. Phase IV mean (±SE) acetaldehyde production (control [solid line], drought [dotted line]) and ethanol (control [long-dashed line], drought [short-dashed line]) in nursery control and drought-treated Douglas-fir seedlings exposed to the indicated temperatures.

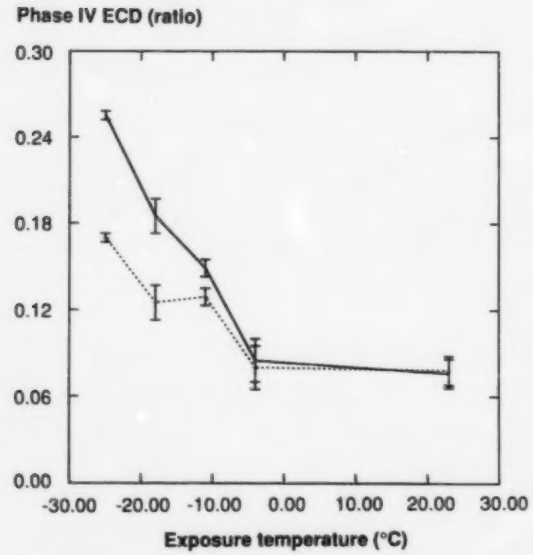

FIGURE 14. Mean (±SE) electrical conductivity damage ratio for nursery control (solid line) and drought-treated (dotted line) Douglas-fir seedlings exposed to the indicated temperatures during Phase IV.

TABLE 11. Regression equation, correlation (r^2), and level of significance (P[F]; P[F]<0.050 is significant) of various parameters during the period of maximum frost hardiness, Phase IV, for control (C) seedlings and nursery drought-treated (D) Douglas-fir seedlings. Variables examined included foliage browning (FB), ethanol (ET), acetaldehyde (AC) and electrical conductivity damage ratio (ECD).

Nursery treatment	Equation	r^2	P(F)	n
C	FB= 0.031(ET) + 0.040	0.676	0.003	10
D	FB= 0.046(ET) + 0.041	0.703	0.002	10
C	FB= 0.041(AC) + 0.055	0.616	0.007	10
D	FB= 0.056(AC) - 0.013	0.534	0.012	10
C	FB= 4.027(ECD) - 0.261	0.775	0.001	10
D	FB= 7.760(ECD) - 0.625	0.892	0.000	10
C	ECD= 0.007(ET) + 0.080	0.699	0.003	10
D	ECD= 0.005(ET) + 0.083	0.461	0.031	10

4 SUMMARY AND CONCLUSIONS

The SIVE technique has passed its first test, in that the information it provides does not contradict that obtained from any of the more accepted methodologies it was compared to. In fact, SIVE data bore a close similarity to post-treatment Sxs spruce seedling survival levels and, in many instances, correlated significantly with it. Additionally, in Douglas-fir, no seasonal pattern of gas production was defined. However, critical threshold values of ethanol production, indicating a stress event, were postulated and checked against low temperature stress events. The results were not rejected, which is encouraging, and this avenue will be pursued in the future.

While not reported here, preliminary seasonal monitoring has also been initiated on standard nursery culture western redcedar (*Thuja plicata*) and hemlock (*Tsuga heterophylla*). The general trend seen in these was similar to that in Douglas-fir, though redcedar had greater levels of gas production (C.D.B. Hawkins, unpublished data). To increase the utility of the SIVE procedure, experiments similar to those done on Douglas-fir will be conducted on redcedar and hemlock while Douglas-fir baseline data continue to be collected.

The SIVE protocol can be carried out very rapidly compared to other techniques (hours versus days or weeks). However, the number of samples processed will be limited as long as the gas chromatograph (GC) is being used as a multi-gas screening instrument of destructive samples. Non-destructive screening protocols that are based on a single gas must be developed. The best candidate appears to be ethanol (see the discussed, postulated stress threshold values using ethanol). A non-destructive screening test would allow almost unlimited numbers of samples to be analyzed and re-analyzed. At least two screening approaches can achieve this goal.

One, in the laboratory, involves changing the column type and the program on the GC and designing new incubation cuvettes. The other, in a nursery or field situation, could be done with portable breath alcohol analyzer technology (MacDonald *et al.* 1989) and modified laboratory cuvettes. However, as these would be screening tests, a number of seedlings would not fall into the good or bad category, but into the uncertain or "grey" class. These seedlings or seedlots would have to be further tested, not only on a multi-gas GC column and SIVE program, but also with the use of other non-destructive, physiologically based seedling vigor tests (Hawkins and Binder 1990). Once this level of assessment sophistication is achieved, a true quality control program can be initiated, from sowing in the nursery to planting in the field.

The data presented indicate that, although more work is required before all the objectives are achieved, the SIVE technique is going to be a powerful seedling assessment tool in forest regeneration.

5 LITERATURE CITED

BMDP. 1988. BMDP statistical software manual. Vol. 1 and 2. Univ. Cal. Press, Berkely, Cal.

Burdett, A.N. and D.G. Simpson. 1984. Lifting, grading, packaging and storing. *In* Forest nursery manual: production of bareroot seedlings. M.L. Duryea and T.D. Landis (editors). Martinus Nijhoff/Dr. W. Junk, Dordrecht, pp. 227–234.

Colombo, S.J. and C. Glerum. 1984. Winter injury to shoots as it affects root activity in black spruce container seedlings. Can. J. For. Res. 14:31–32.

Colombo, S.J. and D.F. Hickie. 1987. A one-day test for determining frost hardiness using the electrical conductivity technique. Ont. Min. Natural Resources, Toronto, Ont. For. Res. Note No. 45.

Colombo, S.J., D.P. Webb, and C. Glerum. 1984. Frost hardiness testing: an operational monitoring manual for use with extended greenhouse culture. Ont. Min. Natural Resources, Toronto, Ont. For. Res. Rep. No. 110.

Drakeford, D.R. and C.D.B. Hawkins. 1989. The stress-induced volatile emissions (SIVE) technique for measuring levels of stress in conifer seedlings. For. Can., Victoria, B.C. FRDA Res. Rep. No. 084.

Duryea, M.L. (editor). 1985. Evaluating seedling quality: principles, procedures and predictive abilities of major tests. Oreg. State Univ., For. Res. Lab., Corvallis, Oreg.

Flint, H.L., B.R. Boyce, and D.J. Beattie. 1967. Index of injury: a useful expression of freezing injury to plant tissues as determined by the electrolyte method. Can. J. Plant Sci. 47:229–230.

Glerum, C. 1985. Frost hardiness of coniferous seedlings: principles and applications. *In* Evaluating seedling quality: principles, procedures and predictive abilities of major tests. M.L. Duryea (editor). Oreg. State Univ., For. Res. Lab., Corvallis, Oreg., pp. 107–123.

Hawkins, C.D.B. and W.D. Binder. 1990. State of the art seedling stock quality tests based on seedling physiology. *In* The target seedling symposium. R. Rose, S.J. Campbell and T.D. Landis (editors). U.S. Dep. Agric. For. Serv., Fort Collins, Col. Gen. Tech. Rep. RM-200, pp. 91–121.

Hawkins, C.D.B. and D.A. Draper. 1988. Height control of interior spruce (*Picea* sp.) by means of photoperiodic induction. *In* Proc. Combined W. For. Nurs. Council, For. Nurs. Assoc. B.C., Intermountain For. Nurs. Assoc. 1988 Meeting. T. Landis (tech. coord.). U.S. Dep. Agric. For. Serv., Fort Collins, Col. Gen. Tech. Rep. RM-167, pp. 45–47.

_____ . 1991. Nursery and field effects following blackout treatment of six spruce seedlots at Red Rock Research Station. For. Can. and B.C. Min. For., Victoria, B.C. FRDA Res. Rep. No. 170. In preparation.

Johnson, J.D. and K.G. Gagnon. 1988. Assessing freezer damage in loblolly pine seedlings: a comparison of ethane production to electrolyte leakage. New For. 2:65–72.

Keates, S.E. 1990. Frost hardiness testing in conifers: a problem analysis. B.C. For. Serv., Victoria, B.C. FRDA Res. Rep. No. 106.

Kimmerer, T.W. 1987. Alcohol dehydrogenase and pyruvate decarboxylase activity in leaves and roots of eastern cottonwood (*Populus deltoides* Bartr.) and soybean (*Glycine max* L.). Plant Physiol. 84:1210–1213.

Kimmerer, T.W. and T.T. Kozlowski. 1982. Ethylene, ethane, acetaldehyde and ethanol production by plants under stress. Plant Physiol. 69:840–847.

Kimmerer, T.W. and R.C. MacDonald. 1987. Acetaldehyde and ethanol biosynthesis in leaves of plants. Plant Physiol. 84:1204–1209.

Kimmerer, T.W. and M.A. Stringer. 1988. Alcohol dehydrogenase and ethanol in stems of trees. Plant Physiol. 87:693–697.

Lavender, D. 1984. Plant physiology and nursery environment interactions affecting seedling growth. *In* Forest nursery manual: production of bareroot seedlings. M.L. Duryea and T.D. Landis (editors). Martinus Nijhoff/Dr. W. Junk, Dordrecht, pp. 133–142.

Levitt, J. 1980. Responses of plants to environmental stress. Vol. I. Chilling, freezing and high temperature stress. Academic Press, New York, N.Y. 495 p.

MacDonald, R.C., T.W. Kimmerer, and M. Razzaghi. 1989. Aerobic ethanol production by leaves: evidence for air pollution stress in trees of the Ohio River Valley, USA. Environ. Pollut. 62:337–351.

Matthews, R.G. 1981. Contrasting approaches to containerized seedling production. 1. British Columbia. *In* Proc. Can. Containerized Tree Seedling Symp. J.B. Scarratt, C. Glerum and C.A. Plexman (editors). Can.-Ont. Joint For. Res. Comm. Proc. O-P-10, pp. 115–122.

_____. 1983. Quality control procedures for container nurseries. B.C. For. Serv., Silv. Branch, Victoria, B.C. File 955-20-1-2. 36 p.

Puttonen, P. 1989. Criteria for using seedling performance potential tests. New For. 3:67–87.

Ritchie, G.A. 1984. Assessing seedling quality. *In* Forest nursery manual: production of bareroot seedlings. M.L. Duryea and T.D. Landis (editors). Martinus Nijhoff/Dr. W. Junk, Dordrecht, pp. 243–259.

_____. 1989. Integrated growing schedules for achieving physiological uniformity in coniferous planting stock. Forestry 62(Suppl):213–227.

Sutton, R.F. 1979. Planting stock quality and grading. For. Ecol. Manage. 2:123–132.

_____. 1988. Planting stock quality is fitness for purpose. *In* Taking stock: the role of nursery practice in forest renewal. C.R. Smith and R.J. Riffle (editors). Ont. For. Res. Council, Toronto, Ont. Symp. Proc. O-P-16, pp. 39–43.

Weiser, C.J., H.A. Quamme, E.L. Proebsting, M.J. Burke, and G. Yelenosky. 1979. Plant freezing injury and resistance. *In* Modification of the aerial environment of plants. Oreg. Agric. Exp. Sta., Corvallis, Oreg. Tech. Paper No. 4734, p. 18.

Wilkinson, L. 1988. The system for statistics. SYSTAT, Inc., Evanston, Ill.

Zaerr, J.B. 1985. The role of biochemical measurements in evaluating vigor. *In* Evaluating seedling quality: principles, procedures and predictive abilities of major tests. M.L. Duryea (editor). Oreg. State Univ., For. Res. Lab., Corvallis, Oreg., pp. 137–141.

APPENDIX 1. Modified GC program for acetaldehyde and ethanol

a) Section 1, GC Control

Heating Program	1	2	3
Oven temperature (°C)	195	210	250
ISO time (minutes)	0	2.5	2.5
Ramp rate (°C•min^{-1})		30.0	30.0

FID sensitivity, High	
Injector temperature (°C)	250
Detector temperature (°C)	300
Flow, 30 mL•min^{-1}	
Carrier gas, He	
Equilibration time	1.0 min
Total run time	6.8 min

b) Section 2, Timed Events

Event		Time
Attenuation	4	-0.30
Relay 0	ON	-0.20
Relay 0	OFF	-0.18
Relay 1	ON	0.01
Relay 1	OFF	0.03
Relay 0	ON	3.10
Relay 0	OFF	3.12

APPENDIX 2. Mean variable values for significant ANOVA factors

	EtOH	Acet	EC_s	FB	BD	CD	SURVIVAL
D 1	2.10	1.05	16.22	0.09	0.31	0.06	0.83
D 2	2.36	2.90	22.76	0.18	0.34	0.09	0.70
D 3	1.77	1.05	25.53	0.15	0.26	0.08	0.77
P 1	1.19	1.32	26.26	0.12	0.36	0.06	0.63
P 2	0.83	0.53	21.02	0.10	0.29	0.03	0.82
P 3	3.27	2.90	18.58	0.13	0.23	0.07	0.84
P 4	3.01	1.93	20.13	0.19	0.32	0.15	0.76
T 1	0.03	0.00	16.00	0.00	0.00	0.00	0.89
T 2	0.45	0.35	18.17	0.06	0.15	0.00	0.83
T 3	5.75	4.65	30.33	0.35	0.76	0.22	0.57

APPENDIX 3. Significant first-order interactions

a)

EC_e	T1	T2	T3
D1	11.19	14.84	22.62
D2	17.56	19.06	31.65
D3	19.26	20.61	36.71

b)

FB	T1	T2	T3
D1	0	0.03	0.23
D2	0	0.11	0.42
D3	0	0.06	0.39

c)

FB	T1	T2	T3
P1	0	0.05	0.30
P2	0	0.04	0.27
P3	0	0.05	0.35
P4	0	0.12	0.47

d)

CD	T1	T2	T3
D1	0	0.01	0.17
D2	0	0.00	0.27
D3	0	0.00	0.24

e)

CD	T1	T2	T3
P1	0	0.00	0.17
P2	0	0.02	0.07
P3	0	0.00	0.20
P4	0	0.00	0.46

f)

SURV	T1	T2	T3
P1	0.67	0.63	0.58
P2	0.93	0.83	0.70
P3	0.97	0.97	0.60
P4	1.00	0.90	0.38

g)

BD	P1	P2	P3	P4
D1	0.32	0.34	0.22	0.35
D2	0.38	0.33	0.27	0.39
D3	0.39	0.21	0.22	0.23

h)

CD	P1	P2	P3	P4
D1	0.02	0.06	0.06	0.11
D2	0.02	0.02	0.07	0.24
D3	0.13	0.00	0.07	0.12

Queen's Printer for British Columbia©
Victoria, 1992

MR 2370